Stimulation of Embryonic Development in *Microplitis croceipes* (Braconidae) in Cell Culture Media Preconditioned With a Fat Body Cell Line Derived From a Nonpermissive Host, Gypsy Moth, *Lymantria dispar*

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A cell culture medium, IPL-52B, was preconditioned with host fat body and two insect cell lines to determine if they would support embryonic development of *Microplitis croceipes* in vitro. The medium was preconditioned with the cell line IPL-LdFB, derived from fat body of the gypsy moth, *Lymantria dispar*, cell line IAL-TND1, derived from imaginal discs of the cabbage looper, *Trichoplusia ni*, and whole fat body tissue from host *Helicoverpa zea*. A second cell culture medium, Excell 400, was preconditioned with only the cell line, IPL-LdFB. Pregerm band eggs were dissected from third instar host larvae and incubated in the conditioned medium for 20 h. Newly laid parasitoid eggs did not develop in unconditioned IPL-52B, but did develop to germ band stage in unconditioned Excell 400. The IPL-52B medium conditioned with both cell lines induced germ band formation, but only the *L. dispar* cell line (IPL-LdFB) promoted significant development to eclosion comparable to host fat body tissue. Excell 400 medium preconditioned with the cell line, IPL-LdFB also supported development to eclosion.

Key words: endoparasitoid, germ band, eclosion

INTRODUCTION

Microplitis croceipes is an important larval endoparasite of both the corn earworm, *Helicoverpa zea*, and the tobacco budworm, *Heliothis virescens*, in

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the Southern United States. It is an effective parasite because it is specific for *Helicoverpa Heliothis* spp., environmentally adaptable, tolerant of certain insecticides, and has a relatively high host search rate [1]. Because of the potential use of the parasite as a biological control agent, developing an artificial rearing medium that would allow the parasite to live outside the host would simplify mass rearing for release in the field. Currently, rearing the parasite in large numbers using the natural host is not economically feasible [2].

Efforts are being made to develop an artificial diet and an in vitro rearing system for *M. croceipes* to allow more economical production of large numbers of this parasite [3]. One difficulty experienced in the in vitro culture of *M. croceipes* eggs is that newly laid eggs must be exposed to a protein normally encountered in host hemolymph to initiate development [4]. It was found that egg development could also be stimulated by exposure to medium preconditioned by fat body dissected from *H. zea* larvae [3,5]. Based upon these findings, we decided to study the possibility of using a fat body cell line from the gypsy moth, *Lymantria dispar*, to promote development of *Microplitis* eggs in vitro. Preliminary studies with the cell line showed that cell-conditioned medium had potential for stimulation of germ band formation in newly laid *M. croceipes* eggs (P. Greany, personal communication). Since the technology for large scale production of this cell line is being developed for use in production of baculoviruses [6], the same technology could be used in mass rearing *M. croceipes*. Therefore, the objective of this study was to determine if the cell line would promote growth and development of the parasite eggs in vitro, similar to use of medium conditioned by host fat body. If so, the cell line might aid in developing a rearing medium for mass production of *M. croceipes*.

MATERIALS AND METHODS

Host and Parasite Colony Maintenance

The host species, *H. zea* (Boddie) was reared as described earlier [7]. The endoparasite, *M. croceipes*, was reared from cocoons purchased from the Delta States Research Center, USDA-ARS, Stoneville, MS. Rearing procedures for *M. croceipes* were as previously reported [8]. Cocoons were held for emergence in environmental chambers at 26 ± 1°C, 70% RH,* and a photoperiod of 15L:9D.

Basal Medium Preparation

Goodwin's IPL-52B medium, without serum or yeastotate [9], was prepared as described earlier [3], and was adjusted to ca. 340 mOsm at pH 6.4. Glutamine was added at 2 mg/ml. Excell 400®[1], a serum-free, prepared insect cell culture medium, was obtained from JRH Biosciences, Woodland, CA.

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*Abbreviations used: D = dark; ETOH = ethanol; L = light; RH = relative humidity; Std. Dev. = standard deviation.

[1]Mention of a proprietary product does not constitute endorsement by the U.S. Department of Agriculture.

Cell Culture

The cell line developed from gypsy moth fat body, IPL-LdFB, was maintained as described by Lynn et al. [10]. A cell line developed from imaginal discs of the cabbage looper, Trichoplusia ni, (IAL-TND1), was maintained as described earlier [11]. Both cell lines were cultured in Excell 400. After cell counts were made with a hemocytometer, the cells were allowed to settle and the medium removed. In one case, the cells were resuspended in IPL-52B, rinsed 3×, and finally resuspended in IPL-52B. The medium was preconditioned with the cells for 20 h at 26.6°C. Total cell counts were made (cells/ml × milliliters of original medium) and the final volume of medium adjusted so that cell concentration equaled 1 × 10^5 cells/ml. Preconditioned medium was centrifuged at 13,000g for 10 min in sterile polypropylene microfuge tubes. Aliquots of (100 μl) supernatant were dispensed into culture dishes (Falcon #1006).

Treatments were as follows: IPL-52B medium preconditioned with IAL-TND1 cells (aggregate form) or host fat body tissue, Excell 400, or IPL-52B medium preconditioned with IPL-LdFB cells, and two control media, made of 100 μl aliquots of each unconditioned medium.

Fat Body Preconditioned Media

Fat body tissue was dissected from nonparasitized fourth instar host larvae and prepared as described earlier [3]. The tissue was rinsed in 0.2 ml of IPL-52B medium to remove contaminants and hemolymph. A total of 20 mg was incubated in 0.1 ml of the two media for 20 h at 26.6°C and 96% RH.

Collection of Eggs and In Vitro Incubation

Parasite eggs were dissected from third instar H. zea larvae that were exposed to 3–5-day-old female wasps for a 2-h period. Host larvae were surface sterilized by momentarily dipping them into 95% ETOH, followed by 5 min immersion in 70% ETOH, after which they were held in sterile distilled H_2O before dissection in IPL-52B. Eggs dissected from host larvae were rinsed 5× in IPL-52B or Excell 400 and pooled before addition of five eggs to each 100 μl drop of conditioned or control medium. Each treatment was repeated 9× for a total of 45 eggs. Means were calculated for each treatment and the standard deviation was used to measure variation of the data. The eggs were incubated at 26.6°C. Development was observed and recorded for 7 days.

RESULTS

The percentage of parasite eggs developing to the germ band stage or actually hatching in IPL-52B medium (unconditioned or conditioned as described above) is shown in Figure 1. Newly laid M. croceipes eggs did not develop in unconditioned IPL-52B (control) but did develop to germ band stage in the unconditioned Excell 400 (control). Most of the eggs incubated in IPL-52B conditioned by H. zea fat body or those incubated in IPL-52B and Excell 400 media conditioned by fat body cells (IPL-LdFB) developed to at least the germ band stage and many hatched. The proportion of eggs attaining the germ band stage and the first instar larval stage in each medium appeared comparable. In contrast, while about 50% of the eggs held in IPL-52B conditioned with imaginal
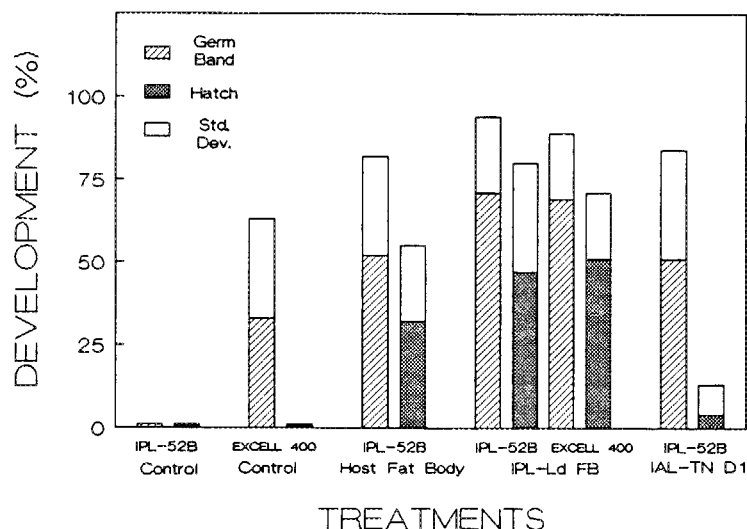
Fig. 1.   Percentage of parasitoid eggs developing to germ band stage and eclosion in IPL-52B and Excell 400 media preconditioned with host fat body, fat body cell line IPL-LdFB, and IPL-52B medium conditioned with wing disc cell line IAL-TND1.

wing disc-derived cell line (IAL-TND1) developed to germ band stage, the number that hatched was low.

Figure 2A–C shows representative individuals derived from each treatment. Figure 2A shows two first instar larvae that developed in IPL-52B conditioned by *H. zea* larval fat body. The first instar larvae with accompanying teratocytes that are typically liberated from the extraembryonic serosal membrane at eclosion, appeared normal in comparison with those dissected from host larvae (not shown). First instar larvae that developed in IPL-52B conditioned by the fat body cell line (IPL-LdFB) are shown in Figure 2B. They appeared similar to those held in media conditioned with host fat body. Thirteen percent of the larvae had enlarged cauda. These larvae, however, were as robust and active as those in host fat body-conditioned medium. Figure 2C shows undeveloped eggs in unconditioned IPL-52B medium and Figure 2D shows eggs that attained germ band stage in unconditioned Excell 400 medium. Although larvae in Excell 400 and IPL-52B media conditioned with the fat body cell line (IPL-LdFB) were similar in size and appearance (not shown), larvae in conditioned Excell 400 were not as active and robust as those in the conditioned IPL-52B medium.

DISCUSSION

Culture media preconditioned with the cell line (IPL-LdFB) derived from gypsy moth fat body stimulated development of *M. croceipes* eggs to eclosion.

Fig. 2.   First instar larvae that developed in IPL-52B medium preconditioned with A: *H. zea* larvae fat body; B: fat body cell line; C: undeveloped eggs in unconditioned medium; D: germ band stage eggs in unconditioned Excell 400 medium.
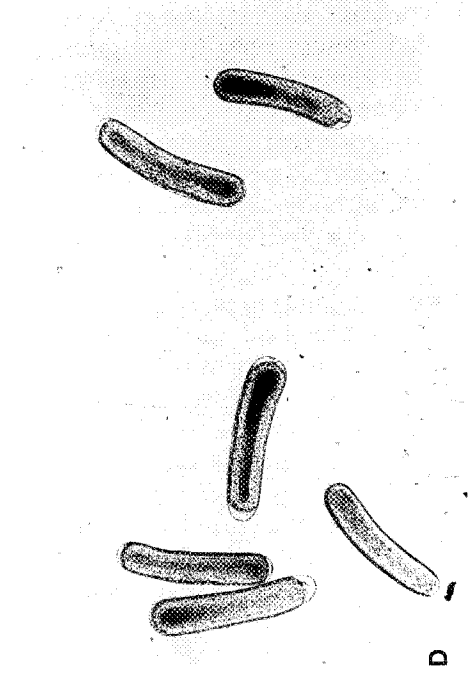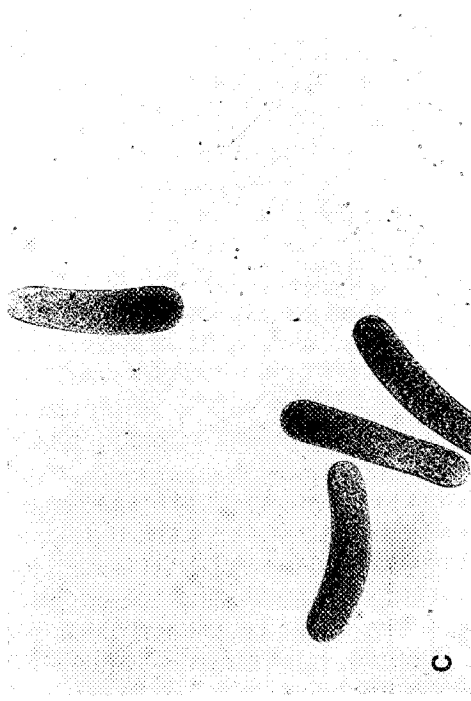
Fig. 2.

In contrast, the cell line (IAL-TND1) derived from imaginal wing discs promoted growth only to the germ band stage. The IPL-LdFB cell line apparently released the required growth factors or other growth supporting substances into the culture medium, in keeping with its fat body origin. A polypeptide that stimulates development of Microplitis eggs in vitro is currently being isolated from host hemolymph [4]. Since the hemolymph bathes the insect fat body, and because the fat body is recognized as the principal biosynthetic source of plasma proteins [11], it seems likely that the fat body is the source of the stimulatory polypeptide. The IPL-LdFB cell line may produce a similar polypeptide or possibly other types of growth-stimulating molecule(s). This finding that a cell line derived from the fat body of a nonhost lepidopteran also can promote M. croceipes egg development shows a lack of obligatory species specificity. These results are in keeping with those reported earlier [3,4] that the hemolymph of four other nonpermissive lepidopteran species stimulated M. croceipes egg development.

That the imaginal disc cell line (IAL-TND1) promoted growth of the pregerm band eggs to the germ band stage shows that there is probably more than one growth-stimulating molecule, including one that stimulates egg development to germ band and others that enable the eggs to complete embryonic development.

Excell 400, a low protein medium, stimulated germ band formation. Because of the propriety nature of the Excell 400 medium, however, it is difficult to suggest what the growth promoting substance might be.

We see two important implications from our finding that the fat body cell line promotes growth of Microplitis eggs in vitro. One is that the cell line might be directly useful in facilitating growth of endoparasites in vitro by providing agents needed by parasite eggs and larvae. A second potential benefit is that the gypsy moth cell line may provide a tool to facilitate isolation of growth factor(s) needed to concoct defined culture media for endoparasites. Little information is available on insect growth factors, despite their potential importance in developing improved culture media for insect cell lines, tissues, and endoparasites [13]. Use of cell line-conditioned media may eliminate the troublesome task of acquiring growth-promoting substances from hemolymph, which has a propensity to melanize.

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